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# SCREENING OF VARIOUS BOTANICAL EXTRACTS FOR ANTIOXIDANT ACTIVITY USING DPPH FREE RADICAL METHOD

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## Abstract

Aiming at the exploration of herbal use by society, crude extracts of the seeds of some commonly used medicinal plants (*Vitis vinifera, Tamarindus indica* and *Glycin max)* were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The overall antioxidant activity of grape seeds (*Vitis vinifera*) was the strongest, followed in descending order by soybean (*Glycin max*) and tamarind (*Tamarindus indica*). The seeds extract of *Vitis vinifera, Glycin max* and *Tamarindus indica* showed 85.61%, 83.45% and 79.26%, DPPH scavenging activity respectively.

**Key words:** Antioxidant activity, DPPH, free-radical, *Vitis vinifera, Glycin max, Tamarindus indica.*

## Introduction

There are many botanical antioxidants, since all plants must protect themselves from oxidation following UV exposure in out-door environment in which they grow. Antioxidant botanicals quench singlet oxygen and reactive oxygen species, such as superoxide anions, hydroxyl radicals, fatty peroxy radicals, and hyperoxides (Jayaprakasha et al., 2001; Nasim et al., 2013; Siddhuraju 2007). Recently, the interest of finding natural antioxidants, especially those of plant origin, has increased greatly (Murtaza et al., 2013; Thornfeldt 2005). Natural antioxidants derived from plants, especially phenolics such as quercetin, carnosol, thymol, catechin, and morin, are of considerable interest as dietary supplements or food preservatives (Sher et al., 2012). In most cases, phenolics mediate their anticarcinogenic effects by inhibiting all stages of chemical carcinogenesis, i.e., initiation, promotion and progression as well as formation of carcinogens from dietary precursors (Guendez et al., 2005; Asad et al., 2012a). Botanical antioxidants have attracted considerable attention because of their skin photo-protective effects (Yilmaz and Toledo, 2006). The antioxidant activity mechanism is predicted or estimated by capturing free oxygen and chelating metal ions. The antioxidant activity is mainly due to flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Shabbir et al., 2012; Slattery et al., 2000). Therefore, recommendations have been made to increase the daily intake of fruit and vegetables, which are rich in these nutrients that lower the risk of chronic health problems associated with diseases (Mahmood et al., 2012).

Grape skins and seeds (*Vitis vinifera*) produced in large quantities by the wine making industry are increasingly used to obtain functional food ingredients (Asad et al., 2012b). Grape seed is a better source of antioxidative constituents than skins of grape/wine byproducts. Functional ingredients of grape seeds include several flavonoids with a phenolic nature such as monomeric flavanols, dimeric, trimeric and polymericprocyanidins, and phenolic acids (Rehman et al., 2012). The antioxidant activity of grape seed phenolic compounds is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders (Kauri and Kapoor, 2001).

Soybean [*Glycine max* (L.) Merr., Fabaceae] is one of the most important crops for human and animal consumption, and the most important organic components of soybean seed are proteins (about 40%) and oil (about 20%) (Khan et al., 2012). Soybean seeds extract is antioxidant, antiproliferative and antiangiogenic, and is used to treat hyperhidrosis in Asian medicines. Epidemiologic studies indicating much lower malignancy and cardiac disease rates in people eating a diet high in soy resulted in thorough investigations revealing its multiple medicinal uses (Razi et al., 2011). The major components of soy are phospholipids (45-60%), such as phosphatidyl choline, and essential fatty oils (30-35%). The minor components include the most active compounds, such as isoflavones, saponins, essential amino acids, phytosterols, calcium, potassium, iron, and the proteases soybean trypsin inhibitor and Bowman-Birk inhibitor. The most potent isoflavones are the phytoestrogens genistein and daidzein (Aqil et al., 2006).

*Tamarindus indica* L. belongs to the Leguminosae family. It is originally from Africa and is grown in humid or arid, tropical and subtropical regions with an average annual temperature of 25°C. It requires great light intensity and is sensitive to cold (Farzana et al., 2011a). It is one of the most important fruits used as spice and food source in Africa [16]. Its sweet and sour pulp and fibrous texture are used for preparing sweets, ice cream, liquors, soft drinks and concentrated juices. Practically, all parts of this plant are used in folk medicine and it has numerous therapeutic applications in humans, including its usage as digestive, tranquiliser, laxative and expectorant (Khalid et al., 2011a). The study of the composition of *Tamarindus indica* L. seeds will bring contribution to health professionals not only regarding adequate dietary orientation but also evaluate its antioxidant potential (Khalid et al., 2011b).

The present study was conducted with the main objective of investigating the most potent antioxidant activity of plant extracts of *Vitis vinifera, Glycin max* and *Tamarindus indica*.

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## Experimental

### Chemicals

1,1-Diphenyl-2-picrylhydrazyl (Sigma, USA), ethanol, methanol, $Na_2SO_4$ and hydrochloric acid (Merck KGaA Darmstadt, Germany), acetone (BDH, England), and n-Hexane (Franken Chemicals, Germany) were purchased through commercial sources. Distilled water was prepared in the Laboratory of Cosmaceutics, from Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan.

### Apparatus

Elisa plate reader (Biotek Synergy HT), electrical balance (Precisa BJ-210, Switzerland), Refrigerator (Dawlance, Pakistan), rotary evaporator (Eyela, Japan), ultraviolet spectrophotometer (Shimadzu Japan), micropipettes, separating funnel, and water bath (Hiha, China) were used.

### Plant Material and Identification

The seeds of grape (*Vitis vinifera*), soybean (*Glycin max*), tamarind (*Tmarindus indica*) were collected from local market of Bahawalpur, Pakistan. The identification of the seeds (*Vitis vinifera)* was performed at the Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan and a voucher specimen was preserved (voucher # GS-LF-8-15-25, GM-SD-7-16-27, and TI- SD-6-15-87) at the herbarium for future reference.

### Extraction Methods

### Preparation of grape seed extract

Grape seed powder (200 g) was extracted with 50% aqueous ethyl alcohol at 60˚C to 70˚C for 2 h. The extract residue was removed by filtration through 16 layers of muslin cloth. The ethyl alcohol was removed under vacuum. The concentrated extract was collected by rotary evaporator and then was filtered through Whatman No.1 filter paper. The extract was stored in refrigerator.

### Preparation of soy bean seed extract

Plant material (1 g per sample) was ground to a fine powder in a mill and extracted for 20 min with ethanol: hexane 1:1 (50 ml) solvent mixture under sonication in an ultrasonic bath at ambient temperature The extract residue was removed by filtration through layers of muslin cloth.The extract was concentrated at rotary evaporator. Concentrated extract was filtered through Whatman No.1 filter paper and was stored extract in refrigerator.

### Preparation of tamarind seed extract

The crushed seeds were extracted with hexane-ethanol-acetone (50:25:25) for 30 minutes, at a 1:3 seed: solvant ratio, under continuous agitation at room temperature. Then the mixture was filtered through layers of muslin cloth and subjected to rotary evaporation under pressure reduced to 40 °C. The extract was concentrated at rotary evaporator. Concentrated extract was filtered through Whatman No.1 filter paper and was stored extract in refrigerator.

### DPPH Preparation and DPPH scavenging activity

1, 1-diphenyl-2-picrylhydrazyl (DPPH) was prepared by taking 1 mg of DPPH in 25 ml methanol to make 100 µl solution (Farzana et al., 2011b). The stable DPPH radical was used for the determination of antioxidant activity. Different concentrations of extracts in respective solvents were added at an equal volume of 10 µl to 90 µl of 100 µM methanolic DPPH solution in a total volume of 100 µl in 96 well plates. The contents were mixed and incubated at 37°C for 30 min. Ascorbic acid was used as standard antioxidant. The reduction in absorbance was measured at 517 nm by using microplate reader; in comparison with the control solution (maximum absorption). The decrease in absorbance indicates increased scavenging activity. The activity is mentioned in percentage form of DPPH radical scavenging according to following formula (Farzana et al., 2011c):
Inhibition (%) = [(Absorbance of control - Absorbance of test)/Absorbance of control] × 100
DPPH radical scavenging activity was measured in triplicate.

## Statistics

At 0.05 level of significance, data were compared to assess significance of difference using SPSS version 17.0.

## Results and Discussion

The antioxidant activity of plants is mainly contributed by the active compounds. Various plants have different free radical antioxidant activity which depends upon their different constituents.

The 50% ethyl alcoholic extract of *Vitis vinifera* seeds showed 85.61% DPPH scavenging activity when ascorbic acid was used as a standard. The antioxidant activity of grape seed extract, primarily resides in the potent antioxidant proanthocyanidins. These polyphenolic bioflavonoids are also known as procyanidins, procyandiol oligomers, leukoanthocyanidins, condensed tannins, and oligomeric proanthrocyanidins (OPCs), and they comprise 60% of poly phenols (Asad et al., 2011). The OPCs consist of dimers of catechins and oligomers of epicatechin and catechin and their gallic acid esters. These compounds are scavengers of both reactive oxygen and nitrogen species (Akash et

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al., 2011). Grape seed also includes other therapeutic compounds including flavonoids such as kaempferol and quercetin glucosides; stilbenes such as resveratrol and viniferins; fruit acids; tocopherols; essential fatty acids; and phenylacrylic acids such as caffeoyl and feruloylsuccinic acid. These compounds are potent antioxidants (Akhtar et al., 2010).

Extracts of soybeans and tamarind showed 83.45% and 79.2% free radical scavenging activity, respectively which were significantly ($P<0.05$) different from each other as well as significantly ($P<0.05$) different from that of *Vitis vinifera*. It has been reported that aceton-water mixture gives better extraction of procyanidins from grape seeds (Ahmad et al., 2008). Researchers have used methanol for qualitative extraction of catechine, epicatechin and epigallocatechin from grape seeds (Luzia and Jorge, 2011). The present study confirms 85.61% antioxidant activity of grape seeds extract when 50% ethanol is used as solvent. It indicates that ethanol water mixture gives better extraction of more total flavonol compounds and procyanidins which are potent antioxidants. The presence of water increases the permeability of seed tissue and thus enables a better mass transport by molecular diffusion (Aqil et al., 2006).

Several bioactive compounds, including phenolics such as phenolic acids, flavonoids and isoflavonoids have been identified in soybeans. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activity of these phenolic compounds (Luzia and Jorge, 2011). Genistein, the major component of soybean isoflavones, has been demonstrated to inhibit ultraviolet -B (UVB)-induced skin tumorigenesis in hairless mice. The antioxidant properties of genistein may explain the mechanisms of its anti-photocarcinogenic action because through either direct quenching of reactive oxygen species or indirect anti-inflammatory effects, genistein was found to substantially inhibit a series of oxidative events elicited by UVB irradiation, including hydrogen peroxide production, lipid peroxidation, and 8-hydroxy-2.- deoxyguanosine formation (Shabbir et al., 2012). It has been evaluated 46.71% DPPH activity of soybean seeds, when the extraction was carried out with 70% aqueous acetone (Kauri and Kapoor, 2001). The present study investigated 83.45% DPPH activity of soybean seeds extract when extraction was done with ethanol: hexane 1:1 solvent mixture. This solvent mixture gives a better extraction of soy isoflavones. *Tamarindus indica* L. seeds are important sources of antioxidant activity as 2-hydroxy-3',4'-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenylacetate and (-)-epicatechin, in addition to oligomeric proanthocyanidins (OPCs) (Akhtar et al., 2010). Luzia and Jorge (2011) reported that ethanolic extract of *Tamarindus indica* seeds shows 75.93% antioxidant activity (Farzana et al., 2011b). In the present study, antioxidant activity (i.e. 79.26%) is evaluated when hexane-ethanol-acetone (50:25:25) mixture is used for extraction of *Tamarindus indica* L. seeds. This solvent mixture gives the more efficient extraction of oligomeric proanthocyanidins (OPCs), catechin and epicatechin type compounds from *Tamarindus indica* seeds, which are important and the most plentiful antioxidants.

**Conclusion**

Recently, much attention has been directed towards extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries. The study shows that there are differences in the contents of antioxidant compounds. Some of the plants can be considered as good sources of natural antioxidants since their extracts were found to possess high antioxidant activity. The highest activity was detected in grape seeds (*Vitis vinifera*) followed by soybean (*Glycin max*) and tamarind (*Tamarindus indica*) seeds respectively. The results of present work indicate that extraction of antioxidants from natural sources by an appropriate solvent mixture is very important in obtaining a fraction with high antioxidant activity.

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